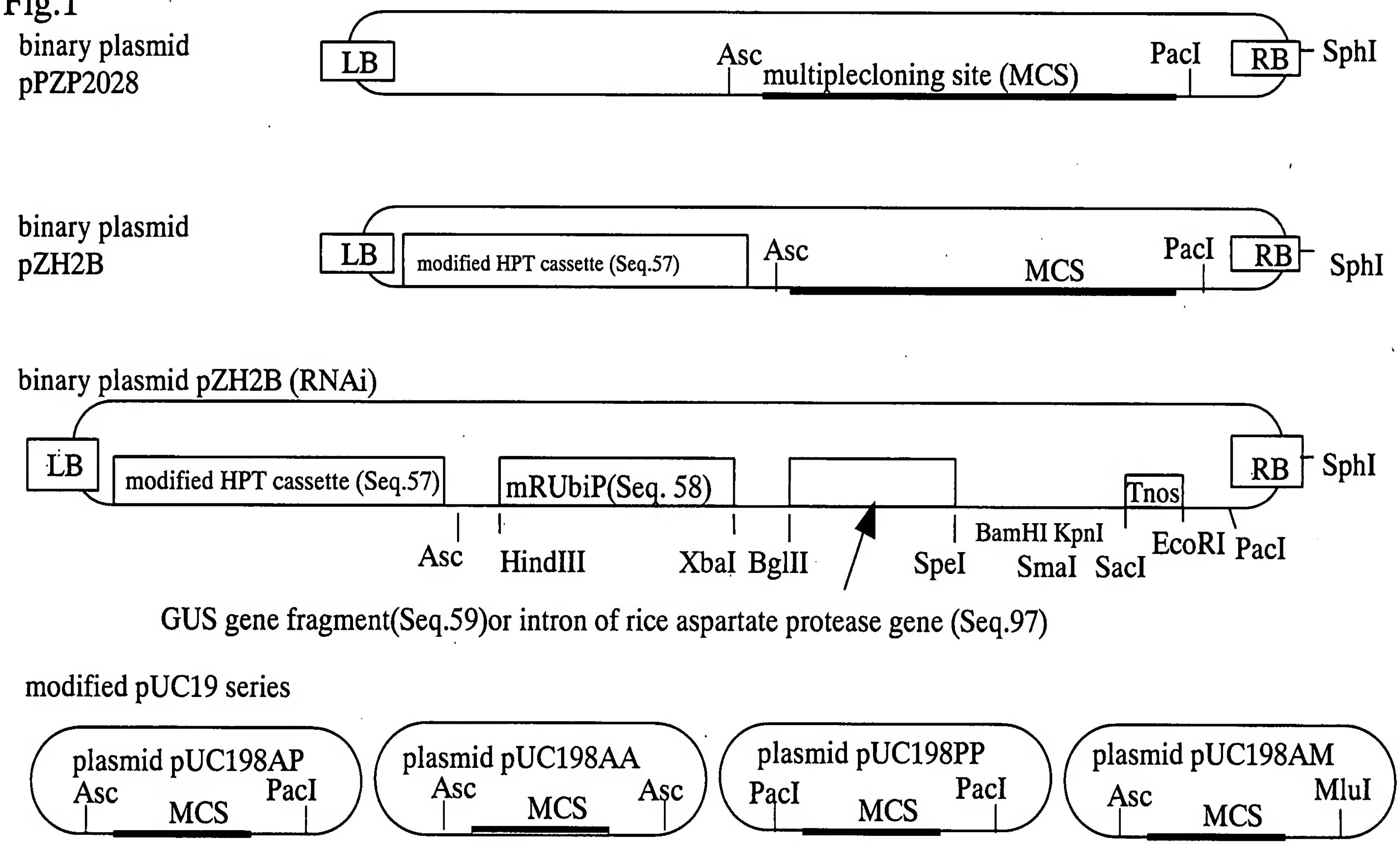
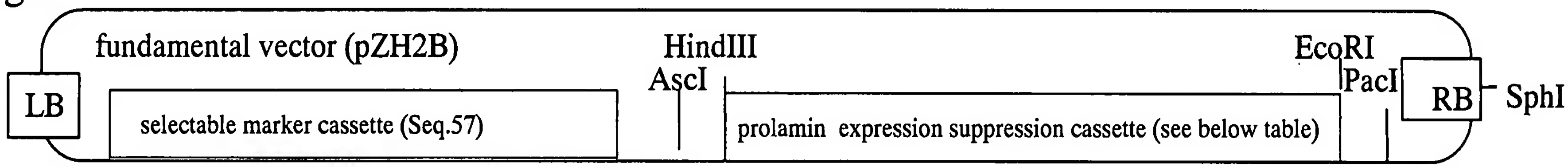
Fig.1



Example of a modified plasmid used for constructing a transgene

**Bold lines indicate multiplecloning sites, having the following restriction sites :
HindIII, SphI , PstI , SalI , XbaI , BamHI , SmaI , KpnI , SacI , EcoRI

Fig.2



A prolamin expression suppression cassette used in a standard antisense method

1) a promoter for expressing a prolamin suppression gene	2) ← prolamin gene fragment	3) terminator
prolamin promoter (Seq.47)	13kDa prolamin(Seq.1)	prolamin terminator (Seq. 61)
prolamin promoter (Seq.47)	prolamin 67bp fragment (Seq.51)	prolamin terminator (Seq. 61)
GluB1 promoter (Seq.48)	13kDa prolamin(Seq.1)	GluB1 terminator
CaMV35S promoter (Seq.49)	13kDa prolamin(Seq.1)	Nos terminator (Seq. 55)

Xba I　Sac I

RNAi type prolamin expression suppression cassette

1) promoter for expressing prolamin expression suppression gene	2)prolamin fragment →	intron (Seq.97)	2) ← prolamin fragment	3) terminator
rice modified polyubiquitin promoter(Seq.58)	13kDa prolamin (Seq.1)		13kDa prolamin (Seq.1)	Nos terminator (Seq.55)
rice modified polyubiquitin promoter(Seq.58)	prolamin15bp fragment (Seq.52,71)		prolamin15bp fragment (Seq.52 , 71)	prolamin terminator (Seq.61)

Xba I　Xba I　Spe I　Spe I　Sac I

The schematic illustration of the structure of prolamin suppression genes indicating exemplary combinations of elements in a expression cassette.

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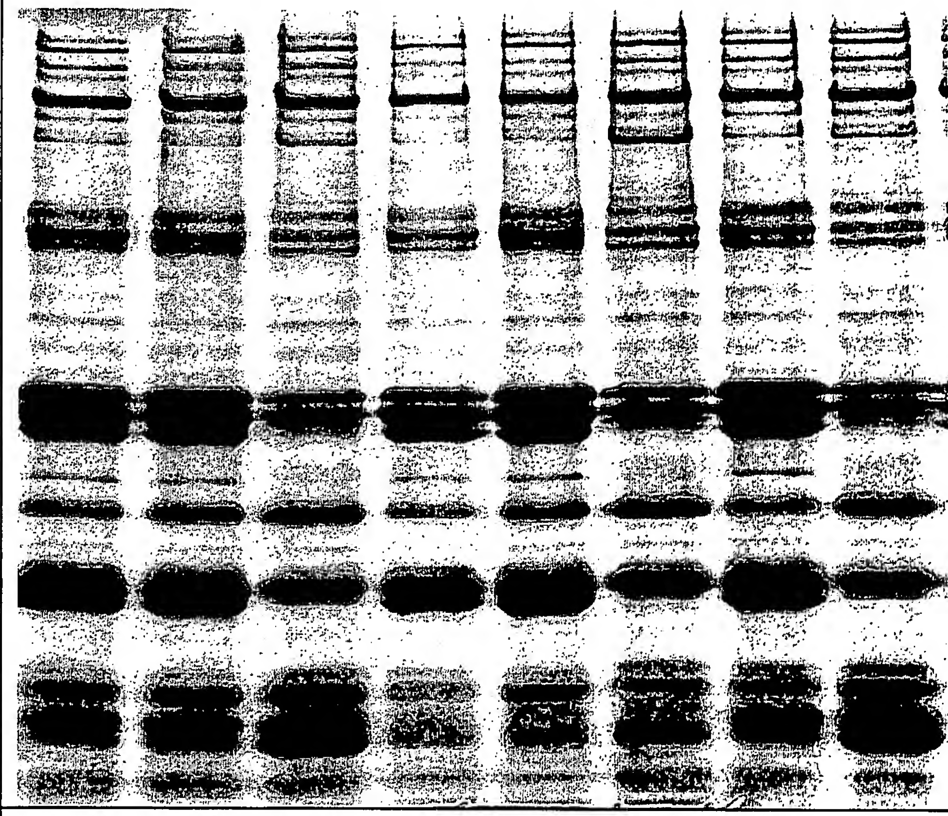
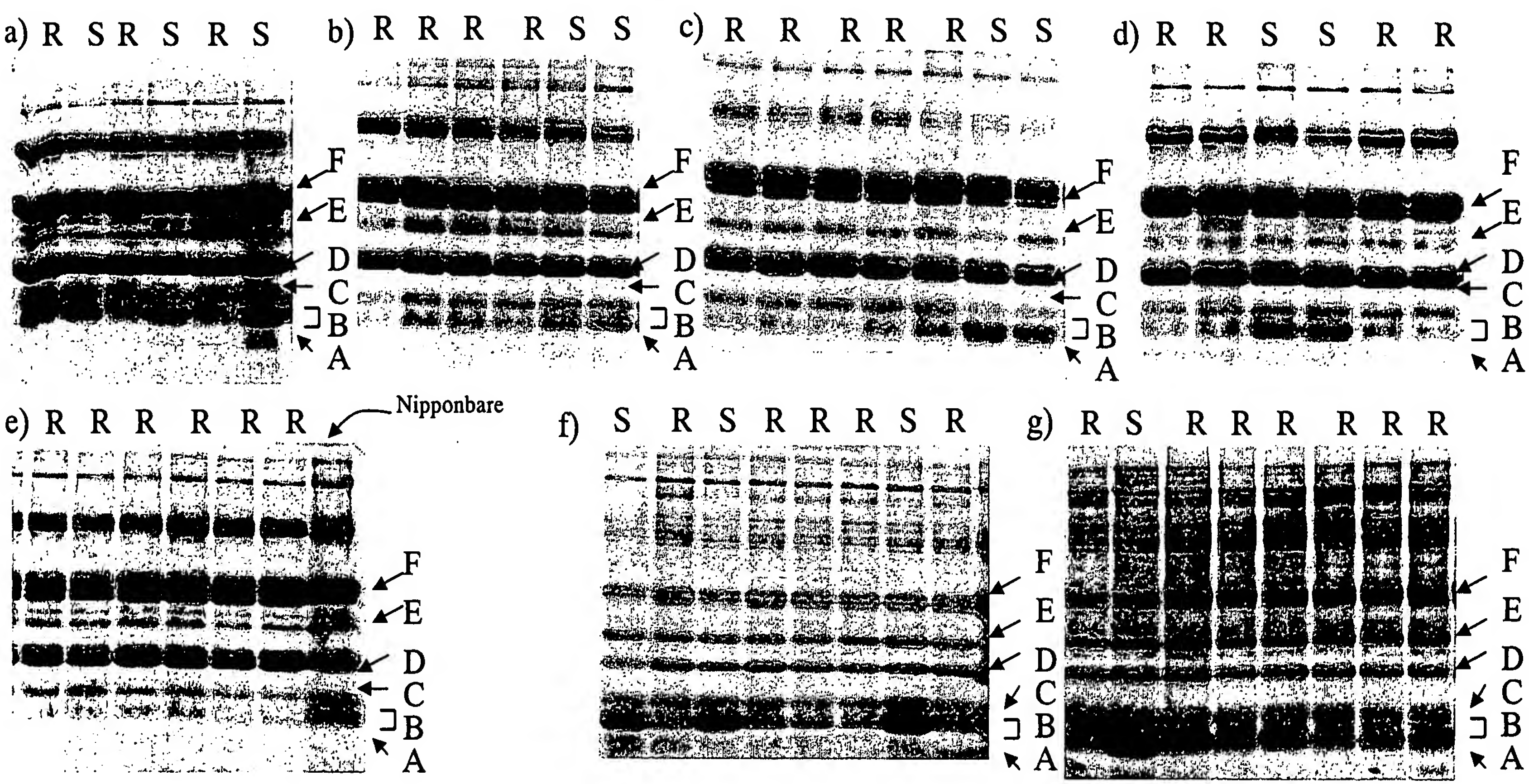
Fig.3



lane	1	2	3	4	5	6	7	8
variety	Nipponbare	Nihonmasari	LGC-1	Nipponbare	Nipponbare	LGC-1	Nipponbare	LGC-1
expression suppression promoter	/	/	/	Seq.47	Seq.47	Seq.47	/	/
Sequences used for expression suppression	/	/	/	Seq.1	Seq.51	Seq.1	/	/

Exemplary SDS-PAGE results of 13kDa prolamin reduced lineage (LP13K)

Fig.4



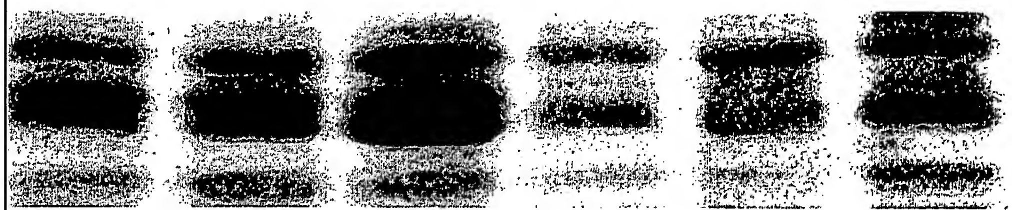
**R:hygromycin resistance, S:hygromycin sensitive

	a)	b)	c)	d)	e)	f)	g)
variety	Nipponbare	Nipponbare	Nipponbare	Nipponbare	Nipponbare	LGC-1	LGC-1
promoter used for prolamin gene expression suppression	Seq.47 (10kDa prolamin)	Seq.47	Seq.48 (glutelinB1)	Seq.47	Seq.47	Seq.47	Seq.47
Sequences used for suppresion	Seq.1	Seq.3	Seq.1	Seq.51	Seq.1	Seq.1	Seq.51
Remark					said a) repeated for three generations		

Fig.5

a)SDS-PAGE

13kDa prolamin



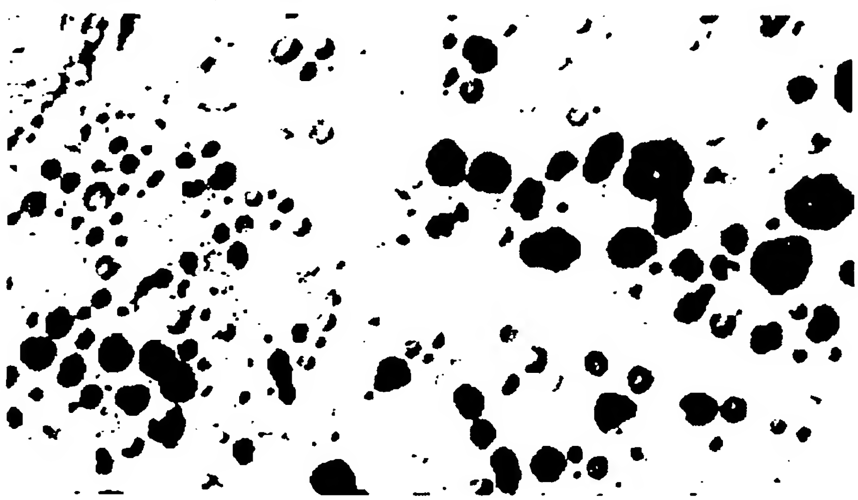
b) Western analysis using anti-13kDa prolamin antibody

13kDaprolamin

lane	1	2	3	4	5	6
variety	Nipponbare	Nihonmasari	LGC-1	Nipponbare	Nipponbare	LGC-1
antisense promoter	/	/	/	Seq. 47	Seq. 47	Seq. 47
Sequence used for antisense	/	/	/	Seq. 1	Seq. 51	Seq. 1
relative values of band concentration in Western analysis	100	100	243	10	25	11

Fig.6a a-1) a variety having 13 KDa prolamin antisense gene

a-2) a standard variety (Nipponbare)



a-3) a variety having reduced glutelin and increased prolamin (LGC-1)

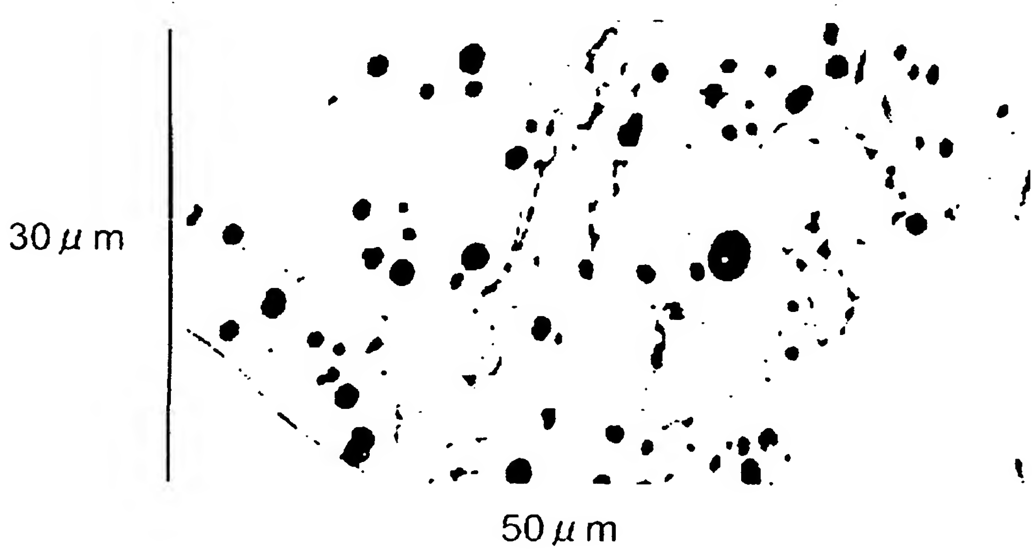
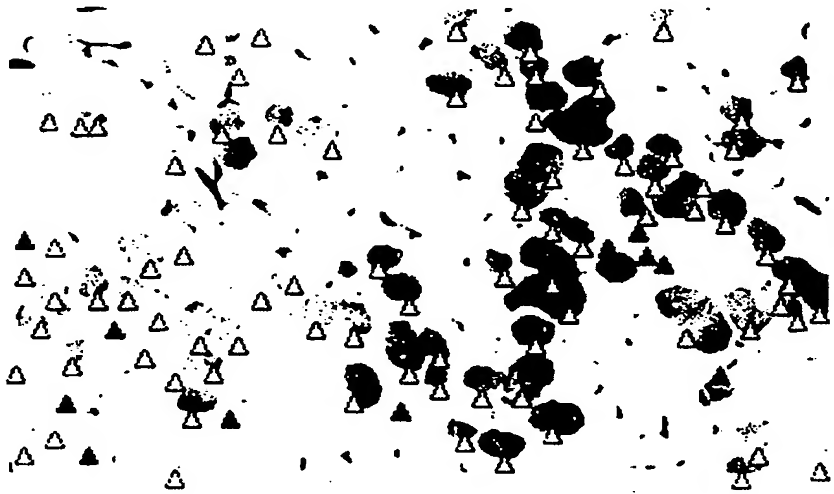
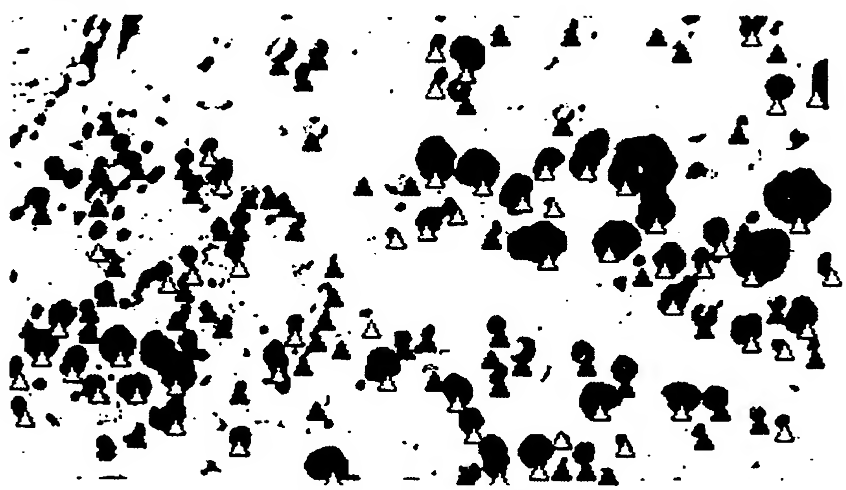


Fig.6b b-1) a variety having 13 KDa prolamin antisense gene



b-2) a standard variety (Nipponbare)



b-3) a variety having reduced glutelin and increased prolamin (LGC-1)

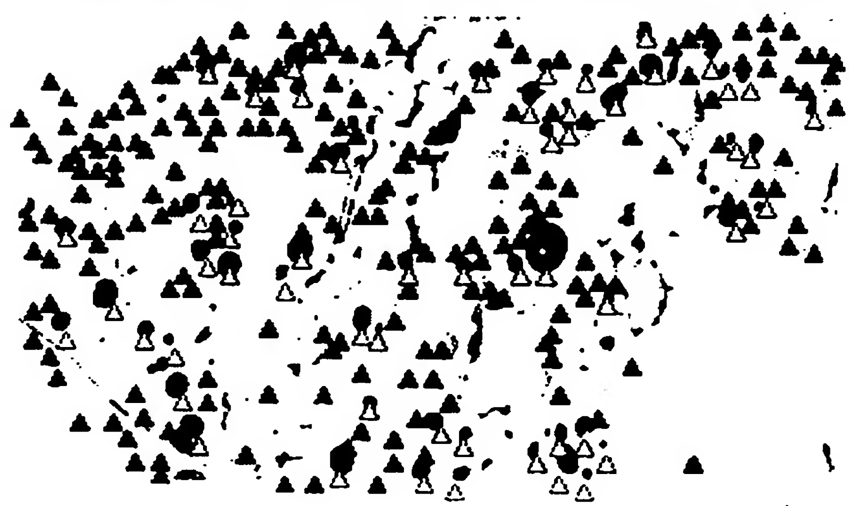


Fig.7

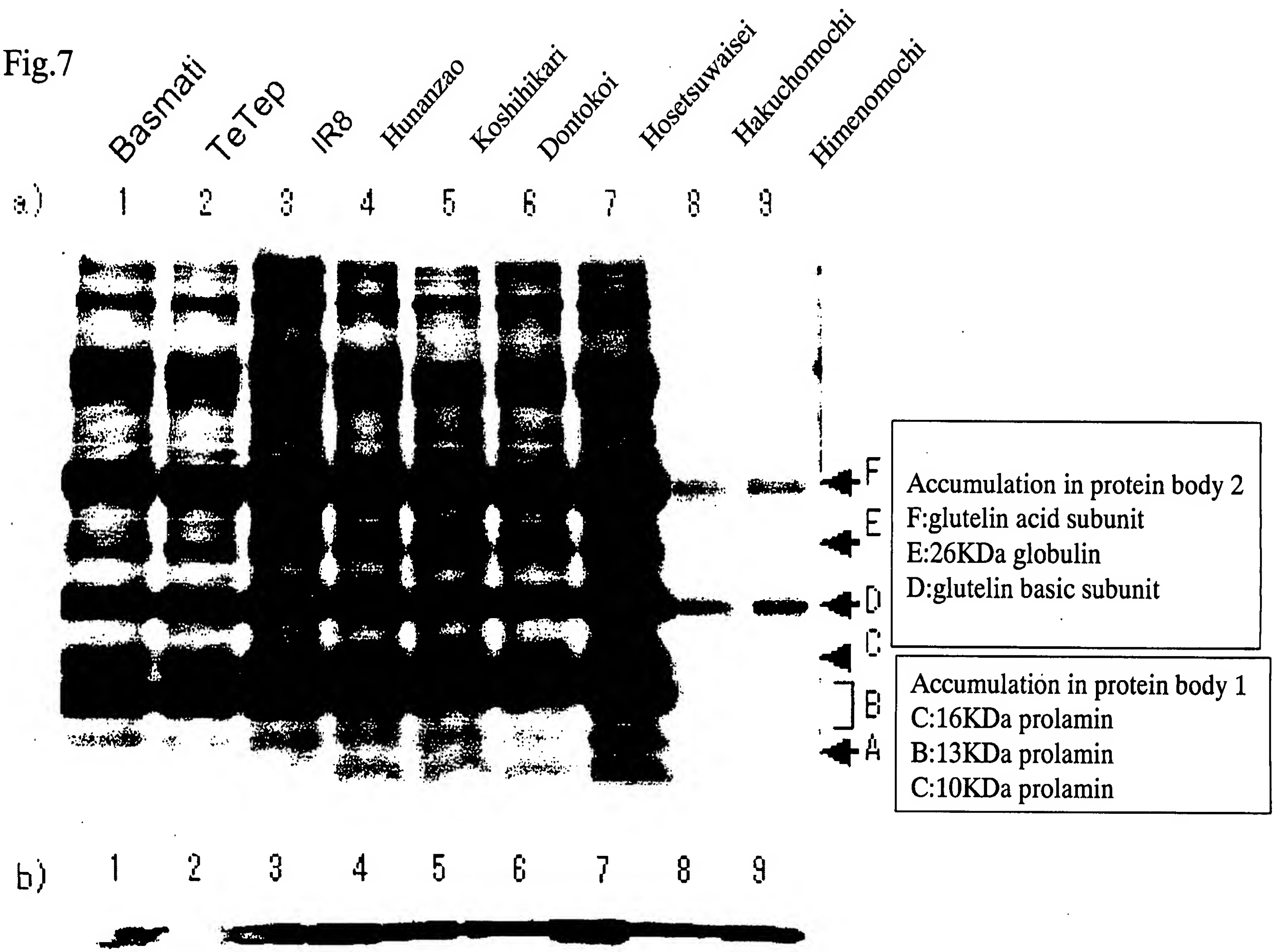

Basmati
TeTep
IR8
Hunanzao
Koshihikari
Dontokoi
Hosetsuwaisei
Hakuchomochi
Himenomochi
a)
1 2 3 4 5 6 7 8 9
F
E
D
C
B
A
Accumulation in protein body 2
F:glutelin acid subunit
E:26KDa globulin
D:glutelin basic subunit
Accumulation in protein body 1
C:16KDa prolamin
B:13KDa prolamin
C:10KDa prolamin
b)
1 2 3 4 5 6 7 8 9

Fig.8

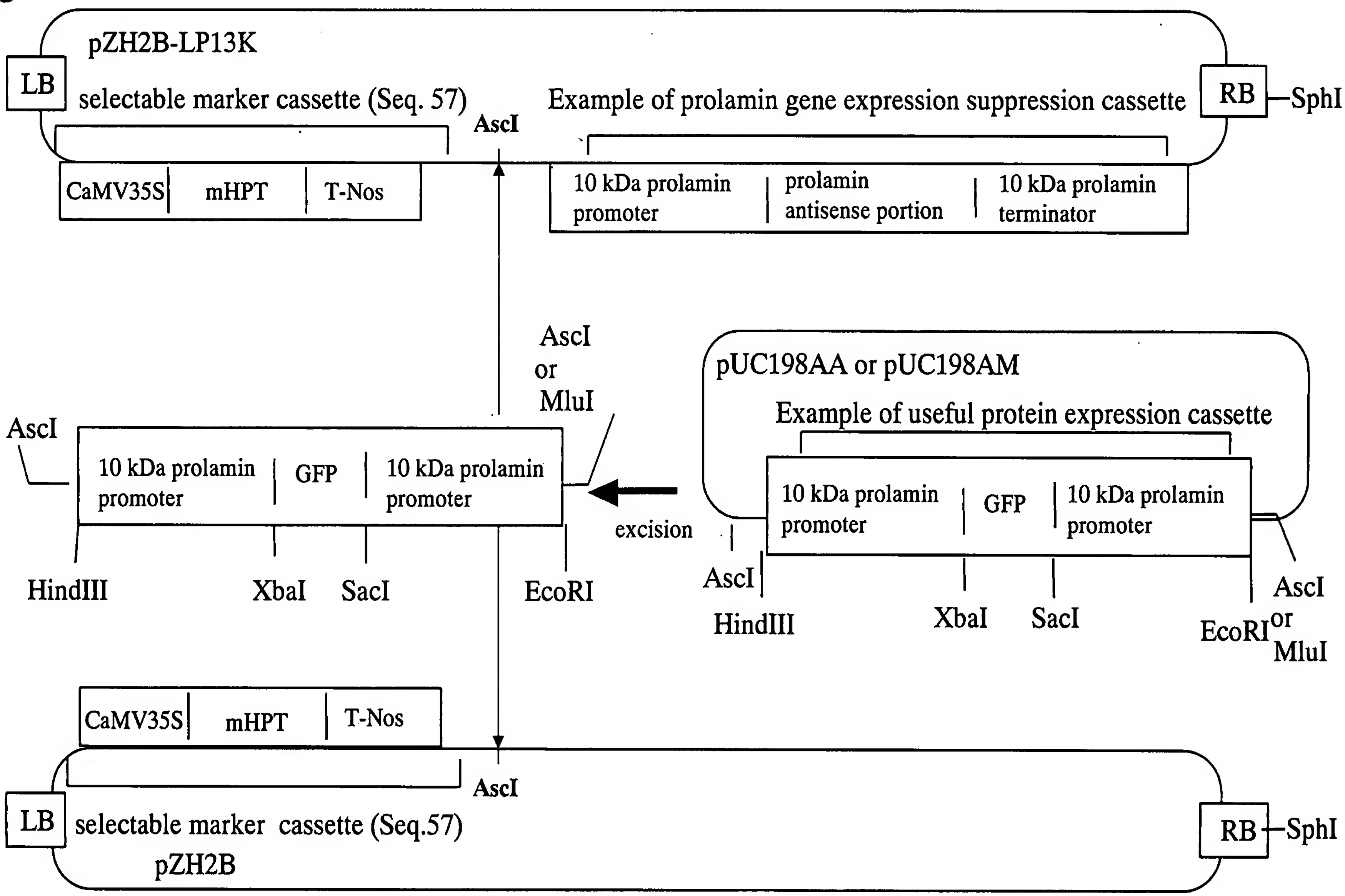


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Fig.9a

Comparative figure of 13K prolamin sequences

```
RM1.NUC       1:---------------AG--GAAGCATAGTAGTAGAATCCTACAAAAATGAAGATCATTTT
RM4.NUC       1:--G--CAAA-ATAGAA--AG-ATC-------TAGTGTCCCGCAGCAATGAAGATCATTTT
RM5.NUC       1:CAATTCAAACATTATAGTTGAAGCATAGTAGTAGAATCCTACAAAAATGAAGATCATTTT
RM7.NUC       1:-------------------GAAGCATAGTAGTAGAATCCAACAACAATGAAGATCATTTT
RM9.NUC       1:--G--CAAA-AGCATA--AG-AAC-------TAGAAACCCACCACAATGAAGATCATTTT
                                    * * *      ***   **   *  ***************

RM1.NUC      61:CGTATTTGCTCTCCTTGCTATTGTTGCATGCAA-CGCTTCTGCACGGTTTGATGCTCTTA
RM4.NUC      61:CGTCTTTGCTCTCCTTGCTATTGCTGCATGCAG-CGCCTCTGCGCAGTTTGATGTTTTAG
RM5.NUC      61:CGTATTTGCTCTCCTTGCTATTGTTGCATGCAA-CGCTTCTGCACGGTTTGATGCTCTTA
RM7.NUC      61:CGTATTTGCTCTCCTTGCTATTGTTGCATGCAATCGC-TCTGCGCGGTTTGATCCTCTTA
RM9.NUC      61:CTTCTTTGCTCTCCTTGCTATTGCTGCATGCAG-TGCCTCTGCGCAGTTTGATGCTGTTA
                * * ******************* ********   ** ***** * *******  * *

RM1.NUC     121:GTCAAAGTTATAGACAATATCAACTACAATCGCATCTCCTGCTACAGCAACAAGTGCTCA
RM4.NUC     121:GTCAAAGTTATAGGCAATATCAGCTGCAGTCGCCTGTCCTGCTACAGCAACAGGTGCTTA
RM5.NUC     121:GTCAAAGTTATAGACAATATCAACTACAATCGCATCTCCTGCTACAGCAACAAGTGCTCA
RM7.NUC     121:GTCAAAGTTATAGGCAATATCAACTACAGTCGCATCTCCTACTACAGCAACAAGTGCTCA
RM9.NUC     121:CTCAAGTTTACAGGCAATATCAGCTGCAGCCGCATCTCATGCTGCAGCAACAGATGCTTA
                 ****  *** ** ******** ** **  *** * ** * ** ********  **** *

RM1.NUC     181:GCCCATGCAGTGAGTTCGTAAGGCAACAGCATAGCATAGTGGCAACCCCCTTCTGGCAAC
RM4.NUC     181:GCCCATATAATGAGTTCGTAAGGCAGCAGTATGGCATAGCGGCAAGCCCCTTCTTGCAAT
RM5.NUC     181:GCCCATGCAGTGAGTTCGTAAGGCAACAGCATAGCATAGTGGCAACCCCCTTCTGGCAAC
RM7.NUC     181:GCCCATGCAGTGAGTTCGTAAGGCAACAGTATAGCATAGTGGCAACCCCCTTCTGGCAAC
RM9.NUC     181:GCCCATGCGGTGAGTTCGTAAGGCAGCAGTGCAGCACAGTGGCAACCCCCTTCTTCCAAT
                ******    *************** ***    *** ** ***** ********  ***

RM1.NUC     241:CAGCTACGTTTCAATTGATAAACAACCAAGTCATGCAGCAACAGTGTTGCCAACAGCTCA
RM4.NUC     241:CAGCTGCGTTTCAACTGAGAAACAACCAAGTC-TG--GCAACA--GCT--C-GC-GCT--
RM5.NUC     241:CAGCTACGTTTCAATTGATAAACAACCAAGTCATGCAGCAACAGTGTTGCCAACAGCTCA
RM7.NUC     241:CAGCTACGTTTCAATTGATAAACAACCAAGTCATGCAGCAGCAGTGTTGCCAACAGCTCA
RM9.NUC     241:CACCCGTGTTTCAACTGAGAAACTGCCAAGTCATGCAGCAGCAGTGCTGCCAACAGCTCA
                ** *   ******* *** ****  ******* **  *** **  * *  *  * ***
```

Fig.9b

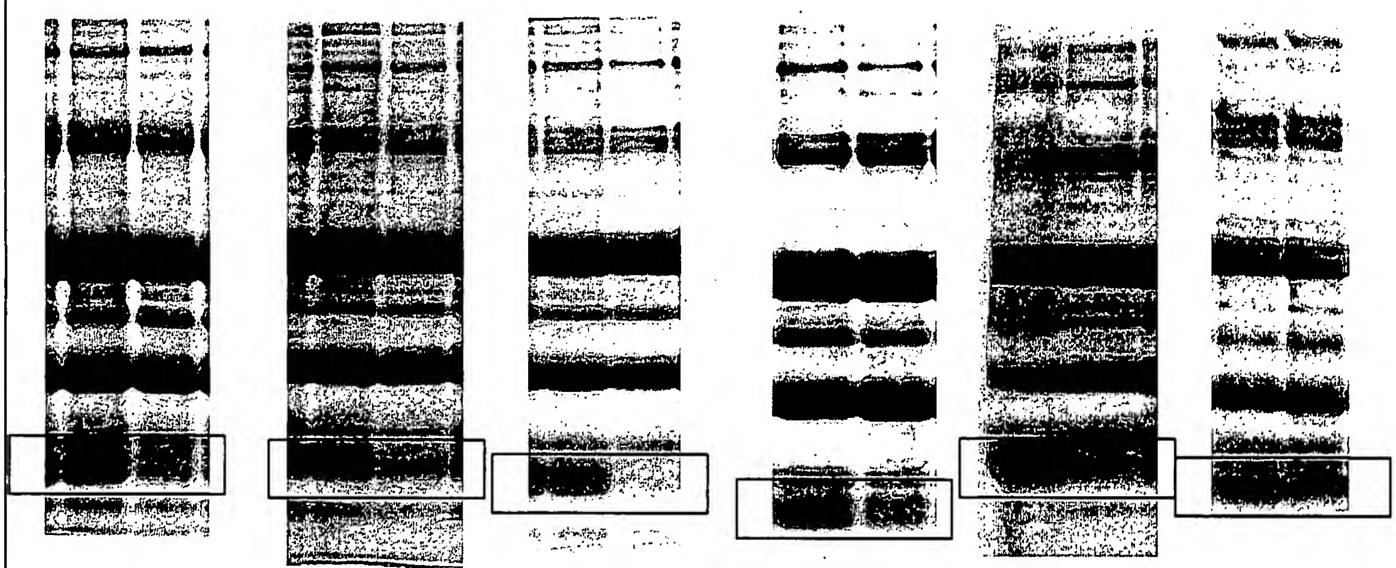
```
RM1.NUC    301:GGCTGGTAGCGCAACAATCTCACTACCAGGCCATTAGTAGCGTTCAGGCGATTGTGCAGC
RM4.NUC    301:GG-TG---GCGCAACAATCTCACTATCAGGACATTAACATTGTTCAGGCCATAGCGCAGC
RM5.NUC    301:GGCTGGTAGCGCAACAATCTCACTACCAGGCCATTAGTAGCGTTCAGGCGATTGTGCAGC
RM7.NUC    301:GGCTGGTAGCACAACAATCTCACTACCAGGCCATTAGTATTGTTCAAGCGATTGTGCAAC
RM9.NUC    301:GGATGATCGCACAACAGTCTCACTGCCAGGCCATTAGCAGTGTTCAGGCTATTGTGCAGC
               ** **   ** ***** *******  **** *****  *  ***** ** ** * *** *
RM1.NUC    361:AACTACAGCTGCAGCAGGTCGGTGTT-GTCTACTTTGATCAGACTCAAGCTCAAGCTCAA
RM4.NUC    361:AGCTACAACTCCAGCAGTTTGGTGATC-TCTACTTTGATCGGAATCTGGCTCAAGCTCAA
RM5.NUC    361:AACTACAGCTGCAGCAGGTCGGTGTT-GTCTACTTTGATCAGACTCAAGCTCAAGCTCAA
RM7.NUC    361:AGCTACAACTGCAGCAATTTAGTGGT-GTCTACTTTGATCAGACTCAAGCTCAAGCCCAA
RM9.NUC    361:AGCTACGGCTACAACAGTTTGCT-AGCGTCTACTTCGATCAGAGTCAAGCTCAAGCCCAA
               * ****  ** ** **  *   *     ******* **** ** **  ******** ***

RM1.NUC    421:GCTTTGCTGGCCTTAAACTTGCCATCCATATGTGGTATCTATCCTAACTACTACATTGCT
RM4.NUC    421:GCTCTGTTGGCTTTTAACGTGCCATCTAGATATGGTATCTACCCTAGGTACTATGGTGCA
RM5.NUC    421:GCTTTGCTGGCCTTAAACTTGCCATCCATATGTGGTATCTATCCTAACTACTACATTGCT
RM7.NUC    421:ACTCTGTTGACCTTCAACTTGCCATCCATATGTGGTATCTACCCTAACTACTATAGTGCT
RM9.NUC    421:GCTATGTTGGCCCTAAACATGCCGTCAATATGCGGTATCTACCCAAGCTACAACACTGCT
                ** ** ** *  * *** **** ** * **  ******** ** *  *** *   ***
RM1.NUC    481:CCGAGGAGCATTCCCACCGTTGGTGGTGTCTGGTACTGAATTGTAATAGTATAATGGTTC
RM4.NUC    481:CCCAGTACCATTACCACCCTTGGCGGTGTCTTGTAATGAGTTTTAACAGTATAGTGGTTC
RM5.NUC    481:CCGAGGAGCATTCCCACCGTTGGTGGTGTCTGGTACTGAATTGTAATAGTATAATGGTTC
RM7.NUC    481:CCCAGGAGCATTGCCACTGTTGGTGGTGTCTGGTACTGAATTGTAACAATATAATAGTTC
RM9.NUC    481:CCCTGTAGCATTCCCACCGTCGGTGGTATCTGGTATTGAATTGTAGCAGTATAGTAGTAC
               **   * * **** ****  * ** *** *** *** *** ** **  * **** * ** *

RM1.NUC    541:AAATGTTAAAAATAAAGTCATGCATCATCATGCGTGAC-AGTTGAAACTTGATGTC-ATA
RM4.NUC    541:GGAAGTTAAAAATAAGCTCAGATATCAT-ATATGTGACATG-TGAAACTT-TGGGTGATA
RM5.NUC    541:AAATGTTAAAAATAAAGTCATGCATCATCATGCGTGAC-AGTTGAAA-AAAAAAA--AAA
RM7.NUC    541:GTATGTTAAAAATAAAGTCATACATCATCATGTGTGAC-TGTTGAAACTTAGGGTC-ATA
RM9.NUC    541:AGGAGAGAAAAATAAAGTCATGCATCATCGTGTGTGACAAGTTGAAACATCGGGGTGATA
                   * ******** ***  ***** * ***** * *****          * *
RM1.NUC    601:TAAATCTAAAT-AAA-C-TCGTGC-C--------
RM4.NUC    601:TAAATAGAAAAAAAGTTGTCTTTCATATTTA---
RM5.NUC    601:AAA-------------------------------
RM7.NUC    601:TAAATCTAAATAAAATCATCTTAC-CTAAAAAA-
RM9.NUC    601:CAAATCTGAATAAAAATGTCATGCAAGTTTAAAC
                **
```

Fig.10

Bands corresponding to 13 kDa prolamin, indicated by boxes →



	10-1	10-2	10-3	10-4	10-6	10-6
Introduction of prolamin expression suppression gene	− +	− +	− +	− +	− +	− +
variety	Nipponbare	Nipponbare	Nipponbare	Koshihikari	Dontokoi	Basmati
13kDa prolamin (relative amount)	100 : 32	100 : 50	100 : 38	100 : 50	100 : 35	100 : 42
promoter used for expression suppression	Seq.58	Seq.58	Seq.58	Seq.47	Seq.47	Seq.47
Sequences suppression and vector types used for expression	RNAi type 45bp(Seq.65)	RNAi type 23bp(Seq.72)	RNAi type (Seq.31)	Seq.1	Seq.1	Seq.1

Fig.11

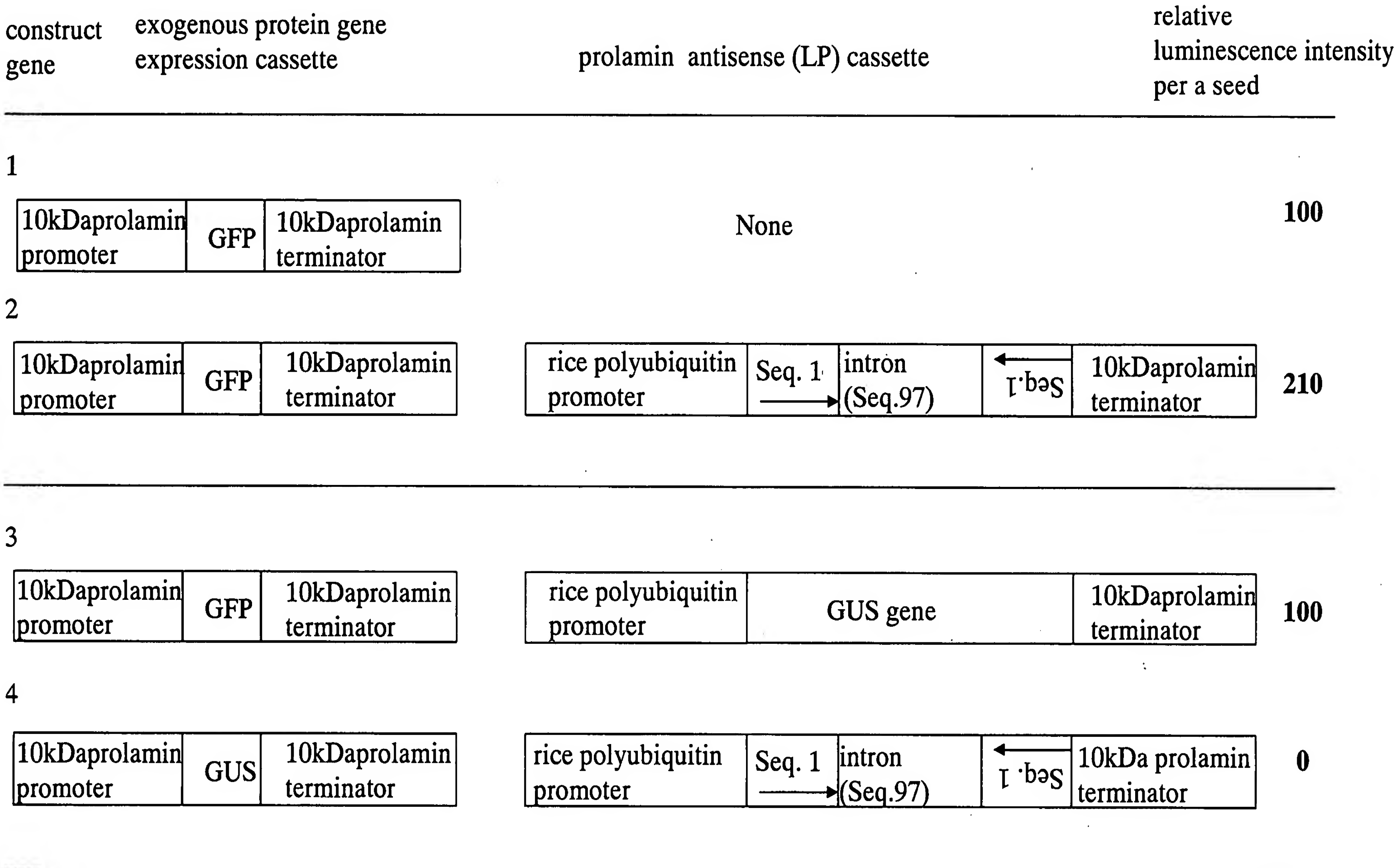

construct gene
exogenous protein gene expression cassette
prolamin antisense (LP) cassette
relative luminescence intensity per a seed
1
10kDaprolamin promoter
GFP
10kDaprolamin terminator
None
100
2
10kDaprolamin promoter
GFP
10kDaprolamin terminator
rice polyubiquitin promoter
Seq. 1
intron (Seq.97)
Seq.1
10kDaprolamin terminator
210
3
10kDaprolamin promoter
GFP
10kDaprolamin terminator
rice polyubiquitin promoter
GUS gene
10kDaprolamin terminator
100
4
10kDaprolamin promoter
GUS
10kDaprolamin terminator
rice polyubiquitin promoter
Seq. 1
intron (Seq.97)
Seq. 1
10kDa prolamin terminator
0

Fig.12

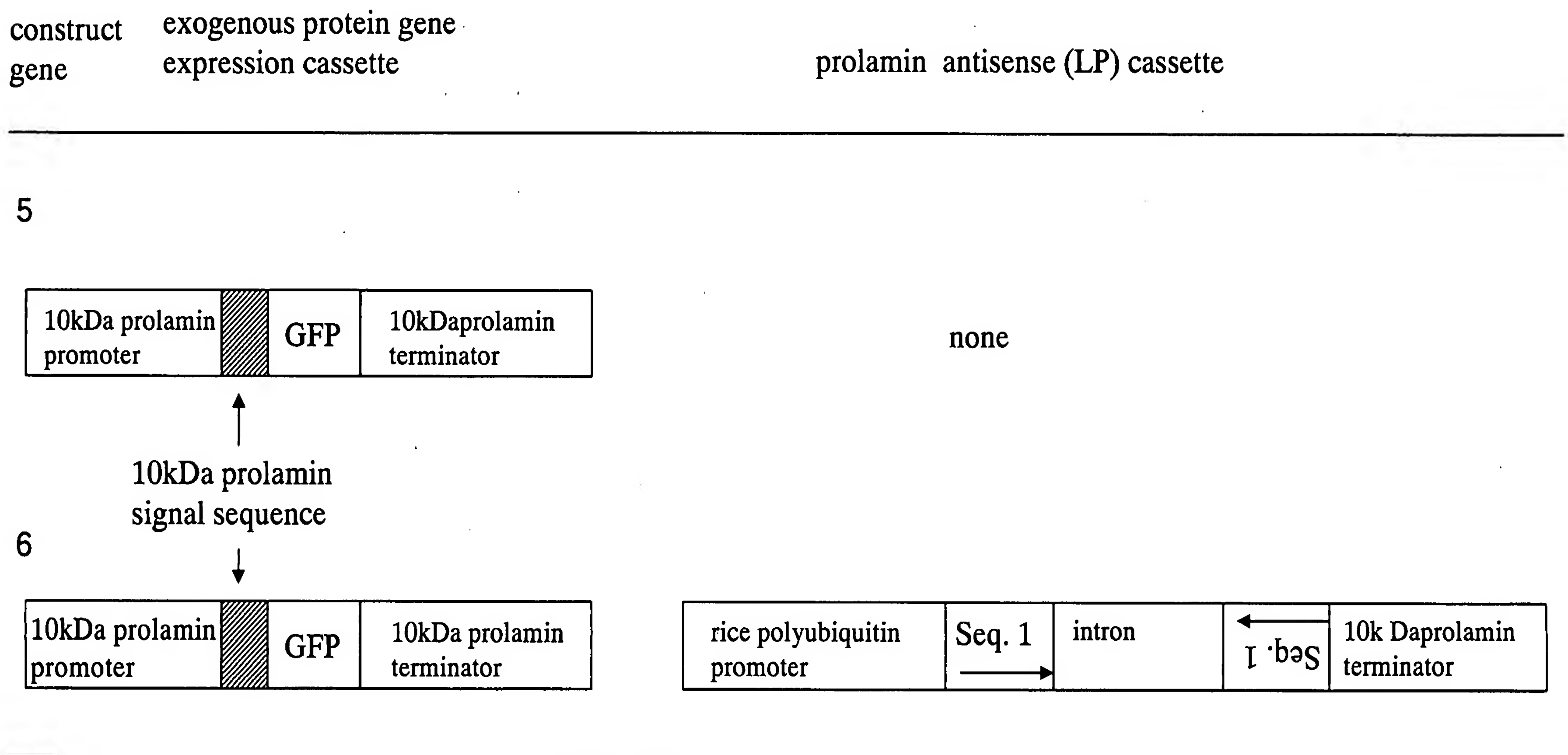
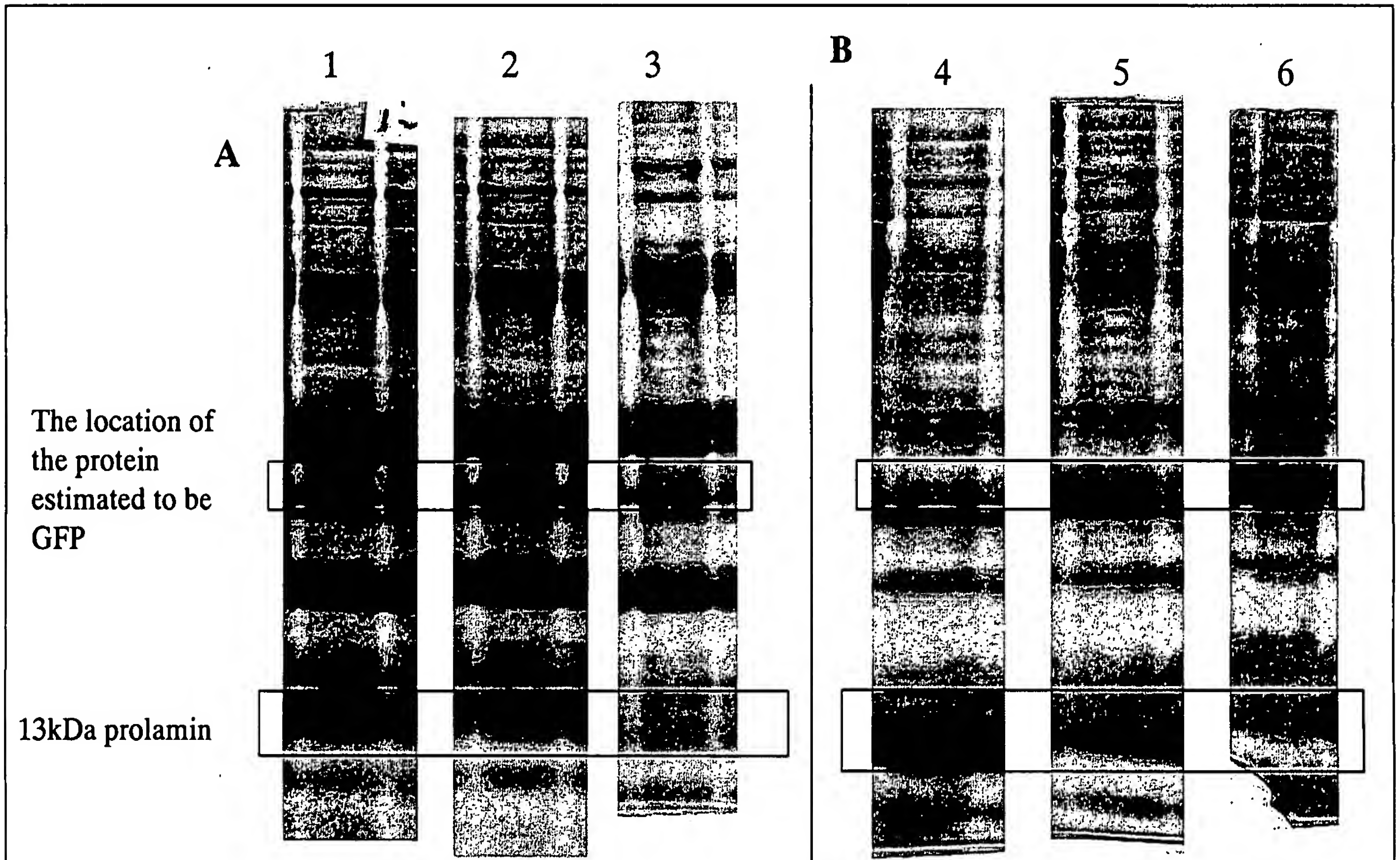

construct
gene
exogenous protein gene
expression cassette
prolamin antisense (LP) cassette
5
10kDa prolamin promoter
GFP
10kDaprolamin terminator
none
10kDa prolamin
signal sequence
6
10kDa prolamin promoter
GFP
10kDa prolamin terminator
rice polyubiquitin promoter
Seq. 1
intron
Seq. 1
10k Daprolamin terminator

Fig.13



variety	Nipponbare	Nipponbare	Nipponbare	LGC-1	LGC-1	LGC-1
transgene	none	gene 5	gene 6	none	gene 5	gene 6
Amount of 13kDaprolamin (relative value)	100	95	15	255	65	25
Amount of GFP (μg/lane)	0	1.9	2.5	0	4.5	5.5

Fig. 14

(A)

ATGGCAGCATACACCAGCAAGATCTTTGCCCTGTTTGCCTTAATTGCTCTTTCTGCAAGTGCCACTACTGCATCTAGAGCAATGGTGAGCAAGGGCGAGGAG
MetAlaAlaTyrThrSerLysIlePheAlaLeuPheAlaLeuIleAlaLeuSerAlaSerAlaThrThrAla<u>SerArgAlaMetValSerLysGly</u>GluGlu

10kDa prolamin signal sequence

ligation sequence site
(restriction enzyme site)

GFP protein

(B)

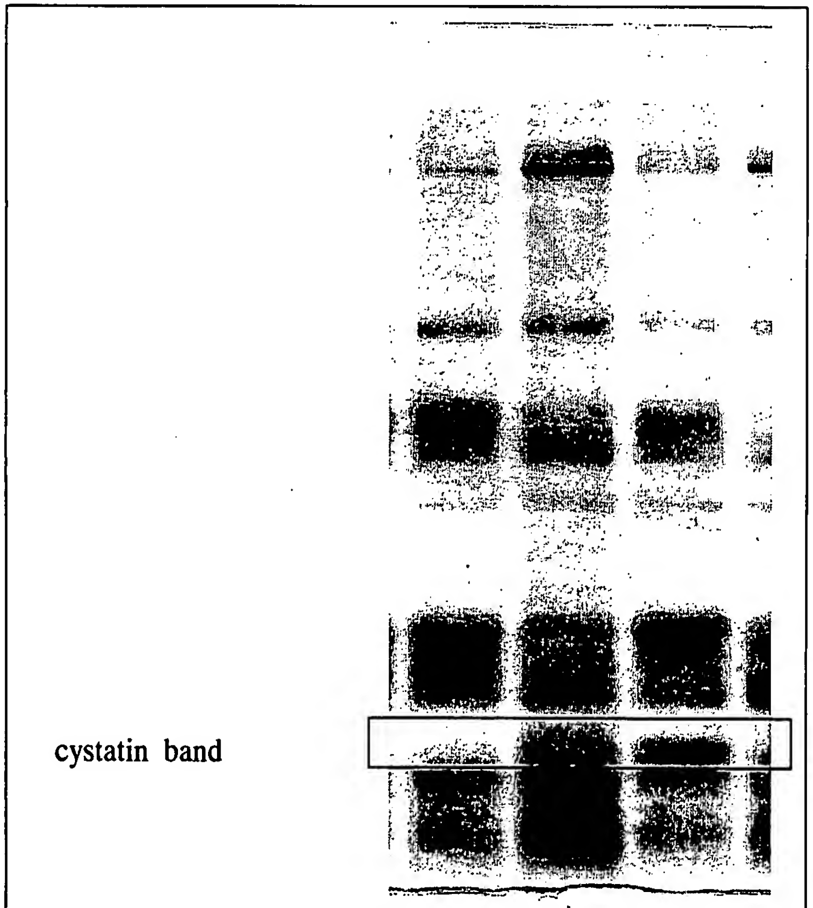
original variety Introducing gene 6 in Fig. 12

1 2

N-terminal sequence (8 amino acid)
SerArgAlaMetValSerLysGly

13kDaprolamin

Fig.15



transgene	none	gene 7	gene 8
Amount of cystatin (μg/lane)	0	0.1	0.4

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Fig.16

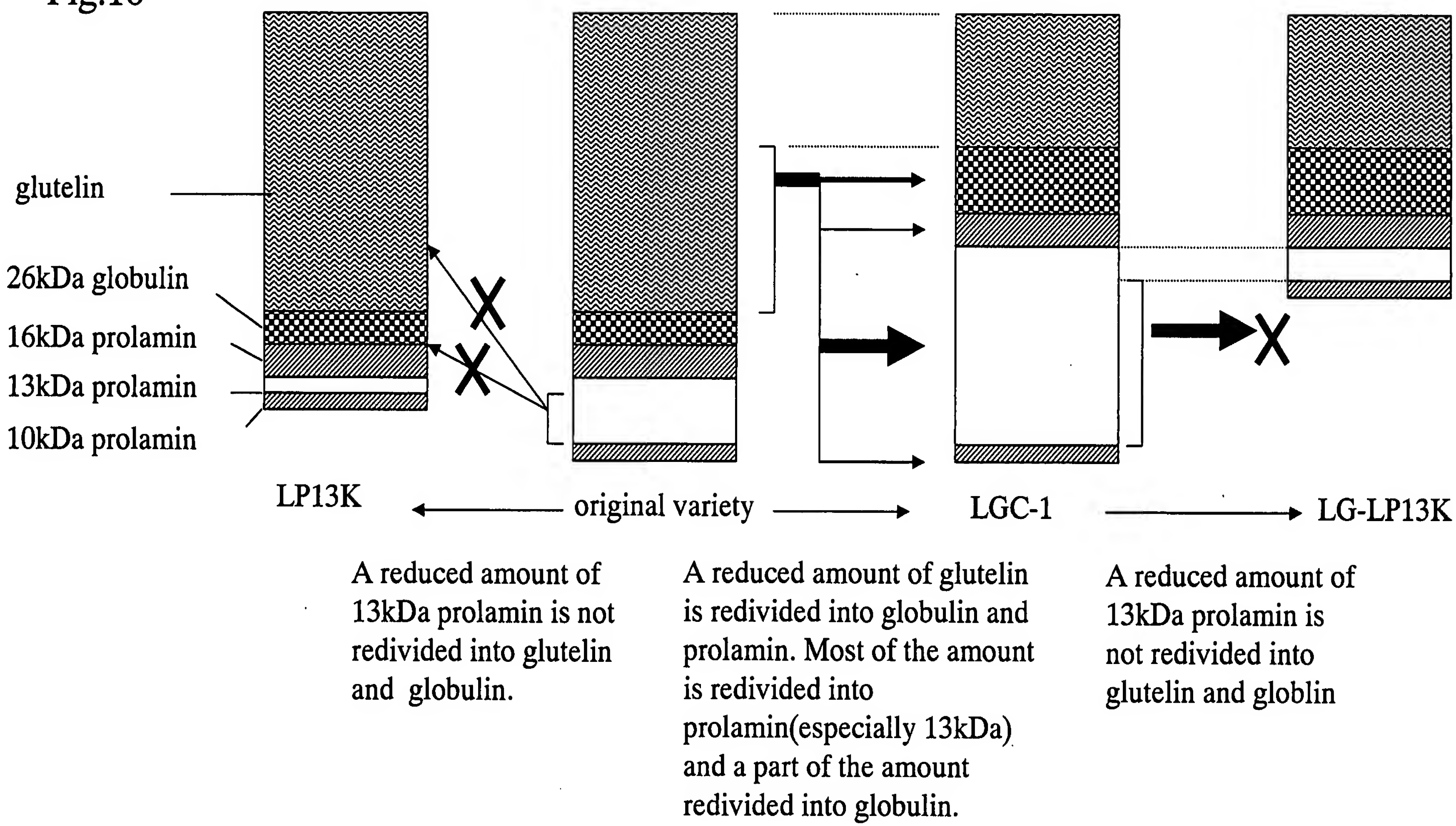
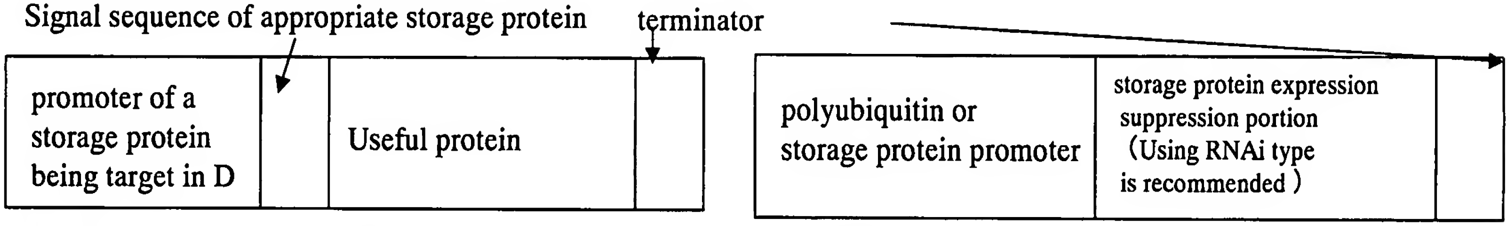


Fig.17 A) crop plant



**Optionally preparing plurality of suppression cassette. In the case, the specific exemplary structure is pursuant to the following B) section

B) rice

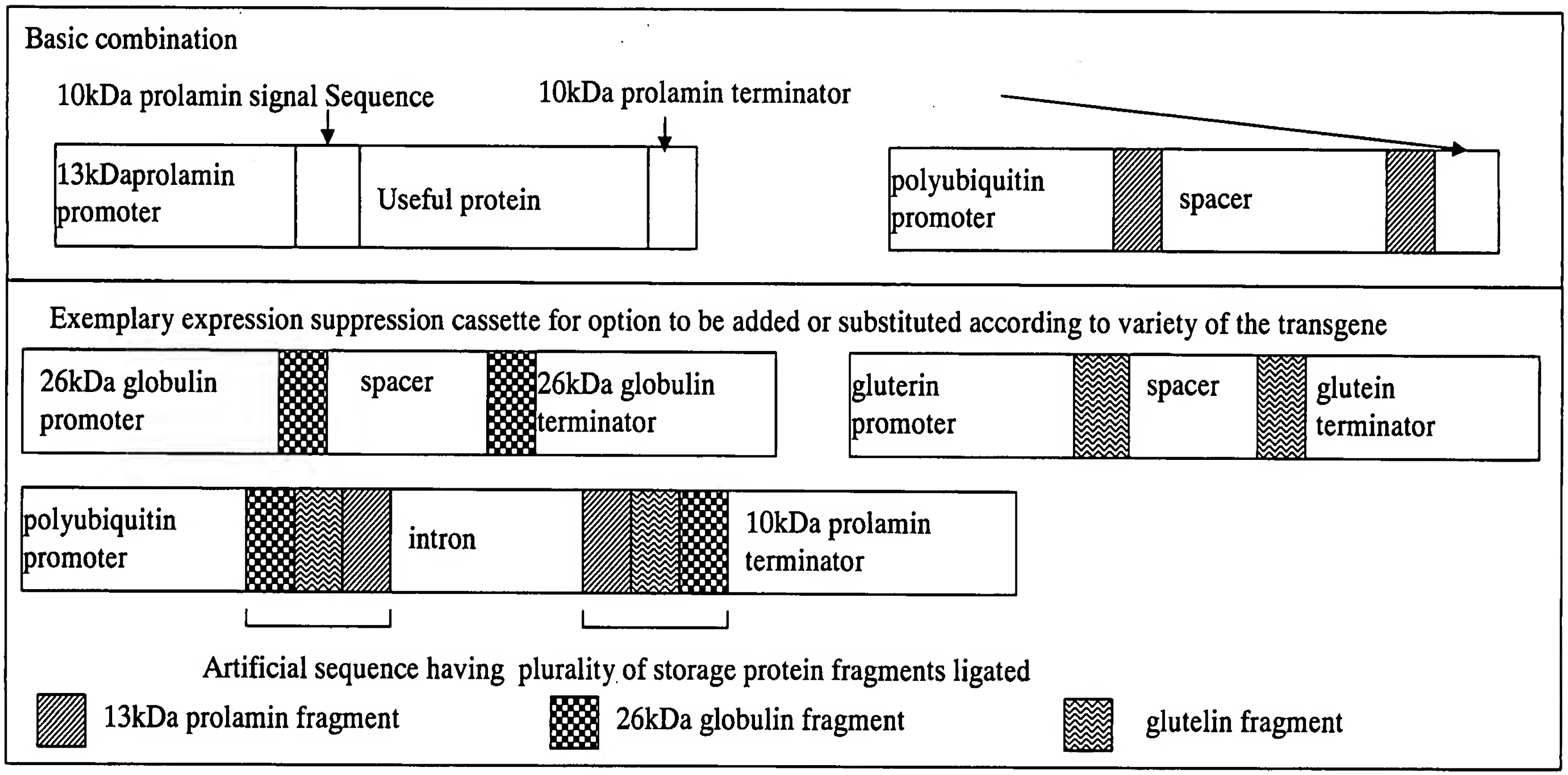


Fig 17 Exemplary structure of expected optimal transgene in using a seed as a bioreactor
Ideally, two or more cassettes are on a fundamental vector